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EDITORIAL COMMENT

Proprotein Convertase Subtilisin/Kexin Type 9 as Transducer of Physiologic Influences on Cellular Cholesterol

A Case for Resistin*

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Circulating cholesterol represents a small fraction (less than 3%) of whole body cholesterol (1,2), and its concentration depends on the balance between lipoprotein output (from liver and intestine) and lipoprotein clearance (mostly by the liver); both processes are regulated at the cellular level. Cholesterol exerts all its functions within cells, serving as a structural element of cellular membranes and a substrate for the production of bioactive molecules such as bile acids, steroids, sex hormones, and vitamin D. Thus, it should not come as a surprise that the regulation of cholesterol fluxes in the body is designed around the needs of individual cells, with the extracellular compartment playing the role of passive reservoir. Each cell is equipped with the machinery to produce cholesterol in a highly synchronized fashion and

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to modulate the intake of plasma-derived cholesterol via regulated expression of the low-density lipoprotein receptor (LDLR). For many years, all the known components of the cellular cholesterol regulation pathways were exclusively intracellular (3). In case of cholesterol depletion, the sterol regulatory element-binding proteins (SREBPs) activate a transcriptional counter-response aimed at increasing synthesis of cholesterol via up-regulation of 3-hydroxy-3-methylglutaryl–coenzyme A reductase and at increasing cholesterol uptake via up-regulation of LDLR. Conversely, in conditions of cholesterol surplus, the SREBP pathway is inactive, and both cholesterol synthesis and LDLR expression are reduced. The discovery of proprotein convertase subtilisin/kexin type 9 (PCSK9), a secretory enzyme that post-transcriptionally regulates levels of LDLR by inducing its degradation, has drastically changed our understanding of the principles of cholesterol homeostasis (4,5). Although synthesized under the regulation of SREBPs and therefore under the control of cellular cholesterol changes, PCSK9 is a protein that is secreted and transported in plasma, thus acting in autocrine, paracrine, and endocrine modes to regulate LDLR levels in ways that may override regulation of cholesterol entry based on the needs of each specific cell (6,7). Therefore, this protein represents the weakest link in the cell-based regulation of cholesterol fluxes because it is exposed to possible regulatory effects of other proteins that may significantly influence its plasma and tissue distribution, its half-life, and its ability to bind to and degrade the LDLR. This "physiological exposure" of the system that regulates cellular cholesterol homeostasis may represent the basis for moderate and severe forms of hypercholesterolemia that are not attributable to genetic causes, a common finding (from 17% to 38% of individuals studied) in cohorts of subjects carrying a dyslipidemic phenotype clinically compatible with familial hypercholesterolemia (8,9). Influences on intracellular cholesterol regulation seem justifiable at the physiological level. For example, it has been proposed that insulin up-regulates the expression of hepatic PCSK9 (10). This regulatory effect of the pancreas over intracellular cholesterol homeostasis could serve the purpose of reducing hepatic lipoprotein uptake after the absorption of a meal, in line with the physiological principle of preferentially distributing chylomicrons to extrahepatic tissues. The effect of insulin on PCSK9 is, however, mediated by SREBP modulation and thus part of an orchestrated choreography of changes in cholesterol-regulating genes. Other influences may be less ontologically justifiable, less physiologically predictable, and less coordinately regulated.

In this issue of the *Journal*, Melone et al. (11) present evidence that resistin, a small protein secreted by cells such as macrophages in humans and adipocytes in rodents (12), increases PCSK9 expression in liver cells and therefore reduces LDLR levels on the cell membrane. Resistin levels are increased in obesity and may contribute to the insulin resistance and low level of inflammation seen in patients with the metabolic syndrome (13,14). The novel mechanism of PCSK9 up-regulation by resistin may contribute to the dyslipidemia commonly observed in obesity, which is often characterized by altered levels of all lipid parameters, including low-density lipoprotein (LDL) (15,16). Indeed, Melone et al. (11) go on to show that the plasma of obese subjects, containing high levels of resistin, had a stronger ability to reduce LDLR on liver cells compared with plasma from lean subjects, containing lower amounts of resistin.

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This effect was abrogated by pre-treatment of plasma with antibodies against resistin. Interestingly, the regulation of PCSK9 by resistin was only partially explained by transcriptional influences. Although there was a definite, 3-fold SREBP-dependent up-regulation of PCSK9, a silencing experiment using small interfering ribonucleic acid against the PCSK9 transcript also suggested significant post-transcriptional regulation, possibly via stabilization of the PCSK9 protein already produced. If these results are confirmed, the implications for our understanding of the physiology of cholesterol homeostasis and for the development of cholesterol therapeutics are very significant, possibly revolutionary. If a regulator of intracellular cholesterol fluxes is influenced post-transcriptionally by circulating proteins unrelated to cholesterol metabolism, then it is possible that the distribution of plasma cholesterol levels in populations reflects, more or less prominently, mechanisms independent from those regulated by established cholesterol genes. Even more profoundly, these results suggest that the tightly regulated machinery in charge of cellular cholesterol homeostasis allows for cholesterol-independent influences from the extracellular milieu to act on the LDLR, the main port of entry into the hepatic cell for circulating cholesterol. An extracellular signal to diminish LDLR levels would be easily defused by the efficient and comprehensive cellular counter-regulatory response, and thus not expected to influence vital cell functions. On the other hand, the reduced cellular uptake of cholesterol would result in its accumulation in the plasma compartment, where no counter-regulatory mechanisms exist.

Even more interestingly, the authors show that not all of the effect of resistin on LDLR was mediated by the up-regulation or stabilization of PCSK9, because a lesser but still significant reduction in LDLR was also seen in cells that do not express PCSK9 (11). Although this may seem confusing and at odds with the hypothesis put forward by the authors (i.e., that resistin acts via PCSK9 to affect LDLR), the result actually represents an intriguing addition to the story line. Because resistin has structural similarity with the cysteine-rich domain in the carboxyl-terminal region of PCSK9 (17), it is easy to speculate that resistin may also interact directly with the LDLR and, as with PCSK9, induce its degradation. The similarity between the 2 proteins in a region such as the cysteine-rich domain, prone to creation of intermolecular disulfide bonding, may also cause resistin and PCSK9 to form aggregation complexes, which may explain the stabilizing effect of resistin on PCSK9 protein when the PCSK9 message is reduced by small interfering ribonucleic acid treatment.

Finally, the influence of resistin on cellular cholesterol homeostasis, either direct or mediated by PCSK9, may explain the wide distribution of LDL-lowering responses to statin therapy in patients. Variability of response to therapy has been shown to be evident particularly in obese subjects (18,19). It is possible that higher levels of plasma resistin in obese individuals may reduce the LDL-lowering effect of statin by amplifying the up-regulation of PCSK9, already known to be an unintended consequence of statin therapy caused by the activation of SREBP (20,21). In the study of Melone at al. (11), liver cells treated with lovastatin showed the expected increase in LDLR, but the addition of resistin increased PCSK9 levels and reduced the statin effect on LDLR both by more than 50%. To make this story more complex, statins are known to down-regulate synthesis of resistin, thus reducing its plasma concentration (22,23). Therefore, "resistance" to statin therapy may be caused not only by an effect of resistin on PCSK9, possibly more prominent in obese subjects, but also by an inability of the statin to down-regulate resistin in the first place. The paper by Melone et al. (11) opens a door into a new world where the regulation of intracellular cholesterol is influenced by circulating proteins apparently unrelated to cholesterol homeostasis. Although direct inhibition of PCSK9 via antibody therapy is currently an active area of investigation (24,25), future intervention may target this protein indirectly via down-regulation or blockade of physiological factors such as resistin.

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REFERENCES

1. Chobanian AV, Hollander W. Body cholesterol metabolism in man. I. The equilibration of serum and tissue cholesterol. J Clin Invest 1962;41:1732–7.
2. Chobanian AV, Burrows BA, Hollander W. Body cholesterol metabolism in man. II. Measurement of the body cholesterol miscible pool and turnover rate. J Clin Invest 1962;41:1738–44.
3. Brown MS, Goldstein JL. The SREBP pathway: regulation of cholesterol metabolism by proteolysis of a membrane-bound transcription factor. Cell 1997;89:331–40.
4. Poirier S, Mayer G, Benjannet S, et al. The proprotein convertase PCSK9 induces the degradation of low density lipoprotein receptor (LDLR) and its closest family members VLDLR and ApoER2. J Biol Chem 2008;283:2363–72.
5. Abifadel M, Varret M, Rabès JP, et al. Mutations in PCSK9 cause autosomal dominant hypercholesterolemia. Nat Genet 2003;34:154–6.
6. Horton JD, Cohen JC, Hobbs HH. PCSK9: a convertase that coordinates LDL catabolism. J Lipid Res 2009;50 Suppl:172–7.
7. Lagace TA, Curtis DE, Garuti R, et al. Secreted PCSK9 decreases the number of LDL receptors in hepatocytes and in livers of parabiotic mice. J Clin Invest 2006;116:2995–3005.
8. Tosi I, Toledo-Leiva P, Neuwirth C, Naoumova RP, Soutar AK. Genetic defects causing familial hypercholesterolaemia: identification of deletions and duplications in the LDL-receptor gene and summary of all mutations found in patients attending the Hammersmith Hospital Lipid Clinic. Atherosclerosis 2007;194:102–11.
9. Chmara M, Wasag B, Zuk M, et al. Molecular characterization of Polish patients with familial hypercholesterolemia: novel and recurrent LDLR mutations. J Appl Genet 2010;51:95–106.
10. Costet P, Cariou B, Lambert G, et al. Hepatic PCSK9 expression is regulated by nutritional status via insulin and sterol regulatory element-binding protein 1c. J Biol Chem 2006;281:6211–8.
11. Melone M, Wilsie L, Palyha O, Strack A, Rashid S. Discovery of a new role of human resistin in hepatocyte low-density lipoprotein

receptor suppression mediated in part by proprotein convertase subtilisin/kexin type 9. J Am Coll Cardiol 2012;59:1697–705.
12. Schwartz DR, Lazar MA. Human resistin: found in translation from mouse to man. Trends Endocrinol Metab 2011;22:259–65.
13. Park HK, Qatanani M, Briggs ER, Ahima RS, Lazar MA. Inflammatory induction of human resistin causes insulin resistance in endotoxemic mice. Diabetes 2011;60:775–83.
14. Reilly MP, Lehrke M, Wolfe ML, Rohatgi A, Lazar MA, Rader DJ. Resistin is an inflammatory marker of atherosclerosis in humans. Circulation 2005;111:932–9.
15. Tershakovec AM, Jawad AF, Stouffer NO, Elkasabany A, Srinivasan SR, Berenson GS. Persistent hypercholesterolemia is associated with the development of obesity among girls: the Bogalusa Heart Study. Am J Clin Nutri 2002;76:730–5.
16. Canoy D, Wareham N, Luben R, et al. Serum lipid concentration in relation to anthropometric indices of central and peripheral fat distribution in 20,021 British men and women: results from the EPIC-Norfolk population-based cohort study. Atherosclerosis 2006; 189:420–7.
17. Hampton EN, Knuth MW, Li J, Harris JL, Lesley SA, Spraggon G. The self-inhibited structure of full-length PCSK9 at 1.9 A reveals structural homology with resistin within the C-terminal domain. Proc Natl Acad Sci U S A 2007;104:14604–9.
18. Ridker PM, Morrow DA, Rose LM, Rifai N, Cannon CP, Braunwald E. Relative efficacy of atorvastatin 80 mg and pravastatin 40 mg in achieving the dual goals of low-density lipoprotein cholesterol <70 mg/dl and C-reactive protein <2 mg/l: an analysis of the PROVE-IT TIMI-22 trial. J Am Coll Cardiol 2005;45:1644–8.
19. Caslake MJ, Packard CJ. Phenotypes, genotypes and response to statin therapy. Curr Opin Lipid 2004;15:387–92.
20. Dubuc G, Chamberland A, Wassef H, et al. Statins upregulate PCSK9, the gene encoding the proprotein convertase neural apoptosis-regulated convertase-1 implicated in familial hypercholesterolemia. Arterioscler Thromb Vasc Biol 2004;24:1454–9.
21. Welder G, Zineh I, Pacanowski MA, Troutt JS, Cao G, Konrad RJ. High-dose atorvastatin causes a rapid sustained increase in human serum PCSK9 and disrupts its correlation with LDL cholesterol. J Lipid Res 2010;51:2714–21.
22. Shyu KG, Chua SK, Wang BW, Kuan P. Mechanism of inhibitory effect of atorvastatin on resistin expression induced by tumor necrosis factor-alpha in macrophages. J Biomed Sci 2009;16:50.
23. Ohbayashi H. Pitavastatin improves serum resistin levels in patients with hypercholesterolemia. J Atheroscler Thromb 2008;15:87–93.
24. Ni YG, Condra JH, Orsatti L, et al. A proprotein convertase subtilisin-like/kexin type 9 (PCSK9) C-terminal domain antibody antigen-binding fragment inhibits PCSK9 internalization and restores low density lipoprotein uptake. J Biol Chem 2010;285:12882–91.
25. Li H, Li H, Ziegler N, Cui R, Liu J. Recent patents on PCSK9: a new target for treating hypercholesterolemia. Recent Pat DNA Gene Seq 2009;3:201–12.

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